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13. ABSTRACT *(Maximum 200 words)*

Genetic screens for new mutations affecting the signaling pathway by which cells respond to TGF-β-related factors in Drosophila were carried out. Two of the genetic strategies employed were unsuccessful and were abandoned. Two other genetic backgrounds that were sensitized using mutations in the Type I receptor gene and in a candidate transcription factor on the signaling pathway have been used successfully to recover new mutations affecting the phenotypes caused by the sensitized genetic backgrounds. The new mutations were recovered at frequencies that are consistent with a limited number of genes (2-3) being present in which mutations can alter the chosen phenotypes. These 10 new mutations are currently being characterized and mapped. Additional genetic screens to recover more mutations are in progress. The genetic mapping of these new mutations will indicate which cloning strategies should be employed for the molecular identification of the affected genes.

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J. Michael Hoff 10.24.95

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION:

The TGF-β growth and differentiation factors have been implicated in the regulation of breast epithelial cell proliferation and in the invasive behavior of metastatic breast cancer (for example: Pierce et al., 1995). The goal of our research is to identify the molecules involved in the signal transduction cascades activated by TGF-β. The initial molecules in the cascade are the TGF-β receptors, transmembrane serine kinases (Kingsley , 1994; Massagué et al., 1994). During the past year several reports have indicated that defects in the type II TGF-β receptor are associated with different human tumors (for example: Markowitz, S. et al., 1995). We predict that other, currently unknown, molecules in the signaling cascades may be affected during tumor progression.

In order to apply a genetic approach to the dissection of this signaling pathway, we have proposed to use the TGF-β-related genes and related receptor genes in the fruit fly Drosophila. Because of the high degree of evolutionary conservation, we predict that the Drosophila genes identified in our screens will provide the necessary molecular probes for the identification of the homologous genes in the human genome. This will permit the analysis of these new genes during the process of breast cancer.

RESULTS:

The strategy proposed in our original application was to identify sensitized genetic backgrounds including mutations in the known genes of the TGF-β-related signaling pathway such that additional mutations in novel genes encoding molecules on the pathway will cause detectable phenotypes. Thus far, we have primarily used mutations in the Drosophila gene thickveins, which encodes a type I receptor essential for signaling by the TGF-b-related Drosophila ligand, dpp. We and others have also identified the Drosophila gene schnurri as encoding a molecule on the signaling pathway (Staehling-Hampton et al., 1995). Drosophila schnurri encodes a large (250kD) protein with seven zinc finger motifs related to the human transcription factors HIV-EP1 and HIV-EP2. Mutations in schnurri disrupt dpp signaling and we are now using schnurri mutations to sensitize the signaling pathway during our screens. During the first year of the project we have tested several different

genetic backgrounds and mutagenesis strategies which are described below.

Screen #1. An F1 screen for second-site non-complementing mutations to the adult viable mutant allele of the receptor gene thickveins.
Mutagen- ethyl nitrosourea
100,000 flies scored
11 new alleles of thickveins recovered
3 second-site noncomplementing mutations mapped to the gene Hairless.

Screen #2. An F1 screen for dominant enhancer or suppressor mutations of the wing venation caused by the hypomorphic thickveins mutant background of tkv5/tkv6.
Mutagen- ethyl nitrosourea or gamma rays
24,000 flies scored
No suppressor mutations recovered
10 flies with enhanced wing vein phenotypes recovered but all were sterile so induced mutations were not recovered

Screen #3. An F2 screen for dominant enhancer or suppressor mutations of the wing venation caused by the hypomorphic thickveins mutant background of tkv5/tkv6.
Mutagen- ethyl nitrosourea
3500 flies scored (second round of similar size currently in progress)
Eight candidate enhancer mutations: five cause lethality, three cause morphological defects.
Seven mutations segregate with chromosome 2; one mutation segregates with chromosome 3
Three of the mutations generate intriguing adult cuticle defects similar to phenotypes observed in flies with mutations in the TGF-β-related gene dpp: defects in distal leg segments and split thorax. The new mutations complement schnurri alleles, however the addition of schnurri mutant alleles into the tkv5/tkv6 genetic background produces similar defects in the legs and thorax.

Screen #4. An F1 screen for dominant suppressors of the wing venation phenotype caused by mutations in both the thickveins receptor and the putative transcription factor on the signaling pathway, schnurri.
Mutagen- ethyl nitrosourea
5000 flies scored (still in progress)

2 flies with putative suppressor mutations

CONCLUSIONS:

Although the first two mutagenesis screens were not successful, the candidates from the third and fourth strategy that are currently being analyzed are promising. These are being mapped by meiotic recombination and complementation tests with known deletions. Further phenotypic characterization of these mutations and localization of the lesions in the genome over the next 2-3 months should permit the initiation of molecular strategies for cloning these genes. This is consistent with the goals and time table initially proposed. We are also continuing to explore other combinations of mutant alleles on the signaling pathway in order to generate additional sensitized genetic backgrounds for further screens.

In summary, our progress thus far is on track with the Statement of Work (Appendix G, pg. 23) of our original proposal. We have made good progress in the first 12 months on Task #1 and are beginning Task #2 on schedule.

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